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A New Preparation Method for Ophthalmic Drug Nanoparticles

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Abstract

The most challenging task in ophthalmic therapy has long been the formulation of suitable ocular drug delivery systems due to the unique structure of the eye that restricts entry of the drug molecule at the site of action. Recently, the use of nanotechnology in the ophthalmic field has gained much attention, since nanoparticulate drug delivery is considered to be one of the most promising technologies to overcome poor drug stability and the difficulties in delivering drugs across biological barriers (improvement of bioavailability). This review demonstrates the usefulness of ophthalmic formulations containing drug nanoparticles. Furthermore, in this review, we introduce a new method established in our laboratory for the preparation of drug solid nanoparticles. This information provides significant information that can be used to design further studies aimed at developing less toxic eye drops.

Keywords: Nanoparticle; Ophthalmic formulations; Corneal toxicity; Transcorneal penetration; Drug delivery

Introduction

The eye is a small and complex organ that is separated from the rest of the body by multiple layers of biological barriers. Moreover, the internal ocular structures and tissues are protected from the external environment by the tight junctions of the corneal epithelium and the mucosal surface. The ophthalmic application of drugs is the primary route of administration for the treatment of various eye diseases, and is well-accepted by patients; however, in traditional formulations, only small amounts of the administered drug penetrate the cornea to reach the desired intraocular tissue due to corneal barriers and dilution caused by lacrimation [1-3]. Consequently, there is a need for the frequent instillation of concentrated solutions to obtain the desired therapeutic effect in both the anterior and posterior hemispheres of the eye. However, the frequent administration of drugs can cause corneal damage as well as undesirable side effects resulting from the systemic absorption of drugs through the nasolacrimal duct [4,5]. Therefore, the primary challenge for ocular drug delivery is how to circumvent these protective barriers in order to achieve therapeutically effective concentrations of drugs in the intraocular tissues. In this regard, it is very important to increase the effectiveness of drugs by enhancing their bioavailability [6]. In order to overcome these problems and increase ocular drug bioavailability, several strategies including the preparation of viscous solutions, micro/nanoparticles and hydrogels have been developed and investigated [1,4,7-10]. In the case of viscous solutions, numerous studies have demonstrated that they do not possess sufficient mechanical strength to resist the ocular clearance mechanism, and offer only a transient improvement in ocular residence time [11,12]. On the other hand, it has been reported that the capability of drugs to penetrate across the cornea can be significantly improved by decreasing the particle size using nanoparticles [3,9,13-15]. Ophthalmic formulations containing drug nanoparticles present a possible solution to the limitations surrounding ocular drug penetration [16-19], and it is known that decreasing direct cellular stimulation and reducing the amount drug used by increasing its bioavailability are useful ways to circumvent the side effects related to drug delivery [6]. It is expected that ophthalmic drug systems using nanoparticles may provide an alternative strategy for increasing ocular drug penetration [16-19]. This review addresses the usefulness of ophthalmic formulations containing drug nanoparticles. In addition, we introduce the new method for the preparation of drug solid nanoparticles.

Design Considerations for Ocular Drug Nanomaterials

The size of a particle influences its functionality in terms of its uptake, residence in circulation, adherence, degradation, as well as clearance [20-24]. The fate of particles inside the body has been reported as follows: ≥ 2 μm, trapped inside liver cells; ≥ 300-400 nm, captured by macrophages and excreted; ≥ 200 nm, filtered in the spleen; ≥ 100 nm, escape from blood vessels through the endothelial lining. Thus, size governs the movement of nanoparticles inside tissues. In the ophthalmic field, nanoparticles in sizes ranging from 10 to 1000 nm allow for the improved topical passage of large, water insoluble molecules through the barriers of the ocular system [25]. Superficial barriers impede direct and systemic drug access to the specific site of action. Drug loaded nanoparticles show favorable biological properties including prolonged residence time for eye drops, decreased toxicity, and increased ability of the drug to penetrate into the deeper layers of the ocular structure and aqueous humor thus minimizing precorneal drug loss caused by rapid tear fluid turnover [6,26]. Techniques were planned to transform nanoparticles from lipophilic to hydrophilic and to down-regulate irritation to the eye. Preparations that include nanoparticles could be very useful for the extended delivery of ophthalmic drugs [2,27,28]. Preparations that include nanoparticles have been used to deliver ocular drugs to target sites in the treatment of many eye diseases as summarized in Table 1 [13,29]. An ideal ocular drug delivery system should possess key properties that include: (I) a controlled and sustained release profile to maintain a therapeutic concentration of the drug over a prolonged period of time to reduce the frequency of administration; (II) specific targeting and prolonged retention in the diseased tissues to improve therapeutic efficiency and mitigate side effects; and (III) patient-friendly delivery routes that minimize or eliminate side effects resulting directly from these administration methods. At present, nanocarrier-based ocular drug delivery systems appear to be the most promising tool to meet the

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primary requirements of an ideal ocular delivery system.

Preparation of Ocular Drug Nanomaterials

Previous research over several decades focused on two major approaches to the design of nanocarriers: bottom-up synthesis and a top-down approach. Bottom-up synthesis, which is based on self assembly and emulsion systems, has been studied extensively in the past, and a variety of potential nanocarriers have been developed using this method, for example, polymeric nanoparticles, micelles, liposomes, nanoemulsions, dendrimers, biodegradable and non-biodegradable carriers, solid lipid nanoparticles, magnetic nanoparticles etc. A majority of these carriers are colloidal systems governed by different forces such as hydrophobic interactions, Van der Waals forces, hydrogen bonding, and ionic interactions. With this approach, high polydispersities are often exhibited, and the systems developed sometimes present certain limitations. In the *in vivo* drug release profiles, the physicochemical characteristics and degradation kinetics of these carriers are difficult to evaluate and reproduce as they are variable. On the other hand, major advancements have been made recently in the fabrication technology by the introduction of the "top-down" approach for micro and nano-fabrication systems using electromechanical techniques. This approach exhibits the potential for designing nanoparticles with precision in terms of particle shape and size. Such an approach can provide control over particle size, functionality, and precise particle geometry. This approach may also resolve the limitations of the bottom-up approach in research.

Eye disease	Drug nanoparticles
Corneal Disease	IM-CS-NPs, CS-CsA-SLN, DS-NS, CS/TCS-SA NPs
Keratoplasty	FK506 PLGA
Corneal neovascularization	Flt23k NPs, Pluronic F 127 diacrylate
Viral keratitis	SLNs, NLCs
Wounds of the corneal epithelium	All-trans retinoic acid NPs
Corneal gene therapy	Chitosan-DNA NPs, deliver plasmid
Corneal tissue engineering	Nanofibrous tissue-engineered scaffolds
Glaucoma	HDNP, CS-HA-NPs, (SLN)NPs
Uveitis	PEG-block-PLA, NP-PEG-TAM
Retinal diseases	Aerosolized NPs, Vacancy engineered mixed-valence-state cerium oxide NPs
Retinal gene therapy	VMD2-eGFP, PEG-POD NPs, Compacted DNA NPs
Age-related macular degeneration	PEG-LPH-NP siRNA, PLA/PLA-PEO NPs
Choroidal neovascularization	bFGF-NPs, (CK30PEG)-compacted DNA NPs

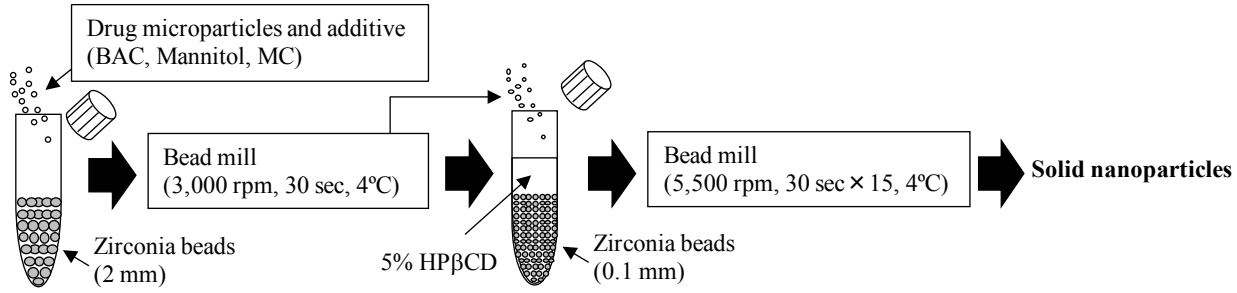
CS, chitosan; CsA, cyclosporine A; HA, Hyaluronic acid; HDNP, hybrid dendrimer nanoparticle; IM, indomethacin; LPH, liposomeprotamine-hyaluronic acid; LC, lipid carrier; NPs, Nanoparticles; NS, nanoparticle suspensions; PEG, poly (ethylene) glycol; PEO, polyethylene oxide; PLA, poly (lactic acid); PLGA, poly (lactic-co-glycolic acid); SA, sodium alginate; SL, solid lipid; TAM, tamoxifen; TCS, thiolated chitosan; VMD2-eGFP, retinal pigment epithelium-specific reporter vector; SLN, solid lipid nanoparticles;

Table 1: Drug nanoparticles in the ophthalmic field.

We have also designed ophthalmic formulations containing drug nanoparticles obtained by mill methods [30,31]. Ophthalmic solutions containing indomethacin (IMC) solid nanoparticles (IMC_{nano}) were prepared using zirconia beads and Bead Smash 12 (a bead mill, Wakenyaku Co. Ltd, Kyoto, Japan) (Figure 1). Briefly, zirconia beads (diameter: 2 mm) were added to IMC microparticles (solid) containing BAC, mannitol or methylcellulose (MC), and the mixtures were crushed with the bead mill for 30 sec (3,000 rpm, 4°C). The mixtures were dispersed in saline with or without 5% 2-Hydroxypropyl-β-cyclodextrin (HPβCD), and crushed again with the bead mill (5,500 rpm, 30 sec×15 times, 4°C) using smaller zirconia beads (diameter: 0.1 mm). The compositions of the dispersions containing IMC are shown in Table 2. 0.5% IMC is equivalent to 14.0 mM IMC; the pH was 6.5 for both ophthalmic dispersions containing IMC micro- or nanoparticles. The IMC particle size reached the nano order by the bead mill method using IMC microparticles containing BAC, mannitol, HPβCD and MC (IMC nanoophthalmic solution, (Figure 2), particle size 76 ± 59 nm, mean ± S.D.). In addition, noprecipitation in the dispersions containing IMC nanoparticles was observed 7 days after preparation. Furthermore, the IMC_{nano} preparations showed high antimicrobial activity approximately equal to that of a 0.001% BAC solution, a preservative used in the ophthalmic field.

Usefulness of Ophthalmic Formulations containing Drug Solid Nanoparticles obtained by Bead Mill Methods

It is very important to elucidate the corneal toxicity and permeability of ophthalmic solutions containingIMC solid nanoparticles (IMC_{nano}). Therefore, we evaluated the transcorneal penetration of IMC_{nano} and its effects on corneal damage using human corneal epithelial cells (HCE-T), and rat and rabbit corneas [30]. The corneal wounds of rat eyes instilled with commercially available IMC eye drops (INDOMELOL® eye drops, Senju Pharmaceutical Co., Ltd, Osaka, Japan) showed 21.6% healing while those instilled with IMC_{nano} showed 48.1% healing 12 hr after corneal epithelial abrasion. In addition, the viability of HCE-T cells



BAC, benzalkonium chloride; HPβCD, 2-hydroxypropyl-β-cyclodextrin; MC, methyl cellulose

Figure 1: Preparation procedures for ophthalmic drug solid nanoparticles by the mill method.

Formulation	Content (w/v%)					Treatment
	IMC microparticles	BAC	D-Mannitol	HPβCD	MC	
IMC_{nano}	0.5	0.001	0.1	5.0	0.5	Bead mill

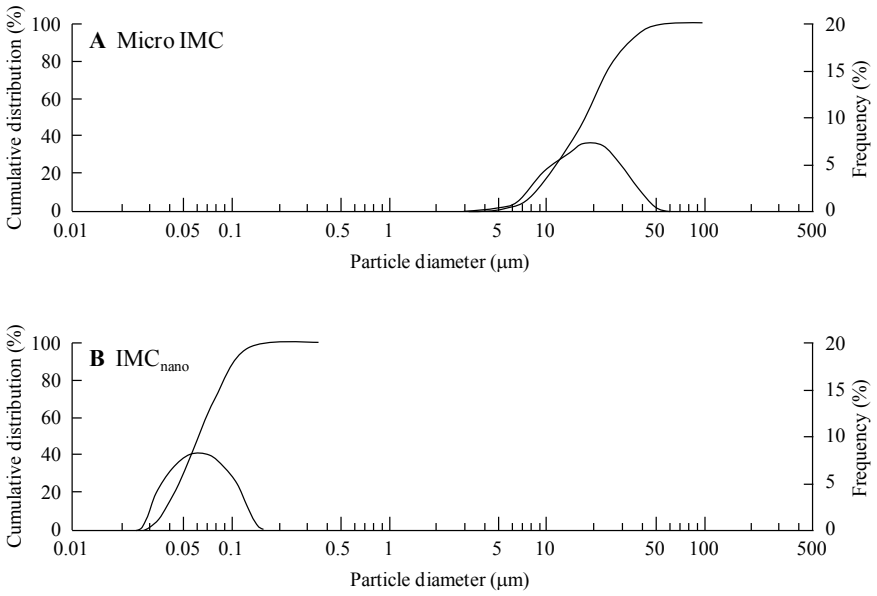


Figure 2: Cumulative size distribution and frequency of 0.5% IMC solid nanoparticles. IMC solid nanoparticleswere prepared as outlined in Figure 1. Particle size was determined using a nanoparticle size analyzer SALD-7100 (refractive index 1.60-0.10i). Particle size: micro IMC, 17.2 ± 12.8 μm; IMCnano,76 ± 59 nm (mean ± S.D.).

treated with IMC_{nano} was significantly higher than that of cells treated with commercially available IMC, pranoprofen (NIFLAN® eye drops, Senju Pharmaceutical Co., Ltd, Osaka, Japan), diclofenac (DICLOD® eye drops, WAKAMOTO Co., Ltd., Tokyo, Japan), bromfenac (BRONUK® eye drops, Senju Pharmaceutical Co., Ltd, Osaka, Japan) or nepafenac (NEVANAC® eye drops, ALCON Japan Ltd., Tokyo, Japan), and the accumulation of IMC in HCE-T cells treated with IMC_{nano} was less than that in cells treated with commercially available IMC. On the other hand, the penetration of IMC from IMC_{nano} was higher than from commercially available IMC eye drops in HCE-T cell monolayers. Furthermore, we have demonstrated the corneal penetration of ophthalmic solutions containing solid nanoparticles using rabbit corneas, which include corneal **epithelial**, stromal and **endothelial** cells (*in vitro* and *in vivo* transcorneal penetration experiments). In the *in vitro* study, the penetration coefficients through the cornea and the cornea/preparation partition coefficients were higher, and the diffusion constants within the cornea lower than those for commercially available IMC eye drops. The IMC penetration rate from IMC_{nano} (18.9 ± 1.95 nmol/cm^2/h, n=5) was significantly higher in comparison with commercially available IMC eye drops (5.43 ± 2.59 nmol/cm^2/h, n=5). In the *in vivo* study, noIMC from commercially available IMC eye drops was detected in the aqueous *humor* until 50 min after administration; however, the lag time from IMC_{nano} eye drops was ca. 25 min. The penetration rate from IMC_{nano} was higher than those from commercially available IMC eye drops, and the penetration rate from IMC_{nano} and the area under the IMC concentration-time curve (AUC_{IMC}) were approximately 10.7 times greater in comparison with commercially available IMC eye drops. These results show that nanoparticle formulations provide a reduction in corneal toxicity, and may make it possible to decrease the amount of drug used *via* an increase in bioavailability, thus resulting in a reduction in drug toxicity.

Conclusion

In this review, we introduce the applications of nanoparticles in the ophthalmic field. Future work should focus on investigating combinations of additives to enhance the favorable properties of nanoparticles. It will be interesting to observe what benefits these innovative drug preparations can provide to patients. In addition, we describe a new method for preparing drug solid nanoparticles established by us [30-32].The particle size of our drug solid nanoparticles is of high quality (particle size, approximately 60-80 nm). Dispersions containing these drug nanoparticles are tolerated better by human and rat corneal epithelial cells than commercially available eye drops, since the accumulation of drug in nanoparticles is lower than from solutions. Furthermore, the state of the dispersions containing drug solid nanoparticles does not affect the antimicrobial activity of BAC against E. coli, and thecorneal penetration of drug solid nanoparticles is significantly better than that from commercially available eye drops. The cost-effectiveness of the new preparation method is also very high, since IMC_{nano} can be prepared in within 2 hour, and this cost for preparation is only reagent cost. It is expected that ocular drug delivery systems using drug nanoparticles may expand their usage for therapy in the ophthalmologic field.

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